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AN INVESTIGATION OF THE POWER OF MESODERMAL DERIVATIVES TO IMMUNIZE MICE AGAINST TRANSPLANTABLE TUMORS

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When the various tissues which have been used to immunize mice against transplantable tumors are passed in review, it is found that representatives of all three germ layers have the power to confer resistance, with the exception of one ectodermal derivative, i.e., the crystalline lens. While both Schöne (1) and Borrel and Bridré (2) have asserted that the injection of testis will not bring about the refractory state, a more recent investigator (3) believes that the testis will confer resistance; furthermore, an experiment conducted in this laboratory by Drs. Bullock and Rohdenburg, and hitherto unpublished, shows that the testis is not without immunizing power, at least in the rat. It has, therefore, been entered in the table (table 3) accordingly (+).

The failure of mouse lens to produce immunity in the experiment of Uhlenhuth and Weidanz (4) is an interesting phenomenon in view of the constancy with which most other tissues call forth the refractory state. Its inactivity cannot be ascribed to an insufficient amount, at any rate, for three doses (1 cc. each) of an emulsion were given; nor was tumor inoculation undertaken at an unfavorable time, for it followed twenty-eight days after the first treatment with lens, and eight days after the last, a period within which immunity should still be high, as Woglom (5) has shown.

The experiment was accordingly repeated by the present writer in another species of animal, rats treated with 0.05 cc.

of an emulsion of rat lens, being inoculated one, two, three, or four weeks afterwards with 0.003 gram[1] of the Flexner-Jobling rat carcinoma by the needle method. The outcome was identical with that recorded by Uhlenhuth and Weidanz, for not the slightest indication of immunity could be discovered in any of the experiments, of which figure 1 reproduces a perfect sample.

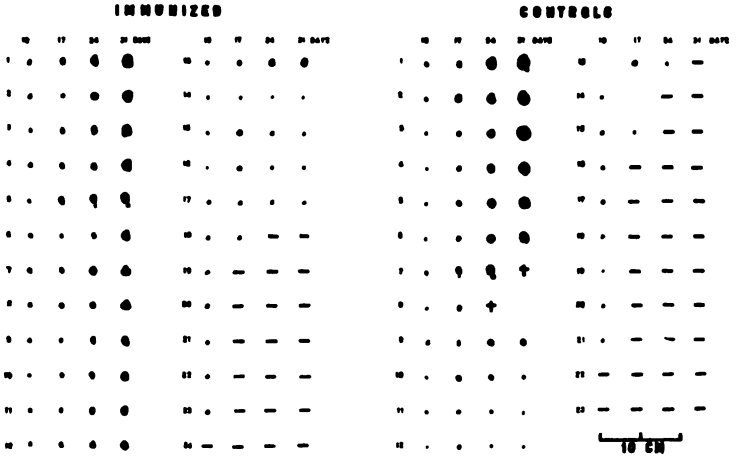

FIG. 1. EXPERIMENT $\frac{\text{FRC}}{19\text{C}}$

No immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of rat lens. Tumor dose, 0.003 gram by the needle method.

It may be objected that the amount of lens administered was too small. But the experience of Bullock and Rohdenburg (6) shows that this quantity of rat embryo skin emulsion will produce a distinct resistance against the same dose (0.003 gram) of the same tumor (Flexner-Jobling rat carcinoma). Still it is possible that the amount of lens, while not actually too small, was relatively insufficient; for the lens contains but a few cells, and the authors (6) just cited have demonstrated that tissues like cartilage and bone, which have a minimum of cellular material

[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method was used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

and a maximum of matrix, do not produce a high resistance. It may be, therefore, that the lens fails for a similar reason, and the comparative failure of all three may perhaps prove to be another vindication of Haaland's assertion (7) that growth of the cells after their introduction is an essential for the production of immunity.

It will be appreciated that the preparation of an emulsion of rat lens in sufficient amount is an expensive and laborious process, and that for this reason a large quantity could not be obtained at the present time owing to war conditions; it may be possible at some future time, however, to repeat one of these experiments, using a larger dose.

The brain will perhaps have to be included among the tissues incapable of eliciting distinct resistance; for, while Borrel and Bridré (8) are said to have succeeded in immunizing with brain, a repetition of the experiment at this laboratory has given entirely negative results. Since the French observers conducted their investigation some time ago, when the importance of dosage and time interval was hardly yet recognized, these more recent experiments, in which both have been taken into account, afford a result that is, perhaps, nearer the truth. Accordingly, the brain has been entered in table 3 as possibly negative, a definite decision, as in the case of the lens, being reserved for the future.

The following experiments were undertaken to determine whether any other tissues share with the lens, brain, cartilage, and bone, their inability to elicit a vigorous immunity. Two mesodermal derivatives—muscle and lymph-node—were chosen. In order that the findings might not be vitiated by the presence of blood in these tissues, the greatest care was taken not to injure large vessels during the removal of the material; the small amount of blood contained in the muscle and the lymph-node themselves may be regarded as insufficient to produce any immunity.

Muscles or lymph-nodes were removed from healthy mice, emulsified, and administered subcutaneously in the left axilla of normal young adult mice in doses of 0.05 cc. (lymph-node) and 0.1 cc. (muscle). Into the opposite axillae of the animals

thus injected, and into untreated normal controls of the same size and breed which had been kept aside under identical conditions, grafts (0.003 gram) of tumor were implanted one to four weeks after the preliminary treatment. The three tumors employed, 63, 11 and 48, are transplantable mammary adenocarcinomata.

Daughter tumors were charted first ten days after the inoculation of the grafts, and subsequently at intervals of one week, and the results thus obtained are reproduced in a number of charts and a table (table 1). These, however, represent but a few of the experiments; it is unnecessary to publish the whole series because the results were similar in all cases.

It will be seen that preliminary treatment with lymph-node calls forth a distinct resistance (figs. 2 to 5) to two carcinomata, though the immunity is perhaps of shorter duration than that induced by some other tissues. The vigorous refractory state obtained with lymph-node, however, obviously has no bearing upon questions regarding the rôle of the lymphocyte in immunity, for an equally high protection can be brought about with other materials.

Muscle, on the other hand, does not induce resistance in the amount employed (figs. 6 to 8). This material, therefore, appears to share with bone, cartilage and the crystalline lens their inability to evoke an efficient immunity against cancer. Why this should be so is not clear; certainly the proportion of cells in muscle is greater than in bone, cartilage, or lens. It may be that the cells of muscle are not readily broken down and absorbed after introduction into the subcutaneous tissues.

The fact having been established that muscle fails to elicit immunity to carcinoma, it becomes necessary to know whether it will confer resistance against a growth which is, like itself, of mesodermal origin, or whether it will resemble such an efficient immunizer against carcinoma as epithelium is known to be, in being unable to produce resistance against the majority of sarcomata. Mice that had been treated with muscle were therefore tested with two sarcomata, the Ehrlich and the Crocker Fund No. 180, both known to be insusceptible to 0.1 cc. of embryo

TABLE 1

CARCINOMA	INTERVAL	MICE	TREATED WITH: Muscle — Survived	Muscle — Negative — Number	Muscle — Negative — Per cent	Lymph-node — Survived	Lymph-node — Negative — Number	Lymph-node — Negative — Per cent
63	1 week	Treated	22	5	22.72	23	21	91.30
		Controls	22	1	4.58	22	1	4.58
	2 weeks	Treated	22	7	31.81	22	18	81.81
		Controls	21	1	4.76	21	4	19.04
	3 weeks	Treated	18	5	27.77	23	7	30.43
		Controls	24	2	8.33	23	0	0.00
	4 weeks	Treated	24	9	37.50	16	3	18.75
		Controls	24	4	16.66	24	2	8.33
11	1 week	Treated	23	6	26.08	10	10	100.00
		Controls	24	8	33.33	10	5	50.00
	2 weeks	Treated	18	6	33.33	20	20	100.00
		Controls	24	6	25.00	22	9	40.00
	3 weeks	Treated	20	7	35.00	9	8	88.88
		Controls	17	8	47.05	12	4	33.33
	4 weeks	Treated	23	7	30.43	22	18	81.81
		Controls	23	10	43.47	24	8	33.33
48	1 week	Treated	23	11	47.82	23	19	82.60
		Controls	22	6	27.27	23	1	4.34
	2 weeks	Treated	21	18	38.09	22	18	81.81
		Controls	23	0	0.00	10	4	20.00
	3 weeks	Treated	19	6	31.59	18	15	83.33
		Controls	21	7	33.33	17	7	41.17
	4 weeks	Treated				19	13	68.42
		Controls				21	3	14.28

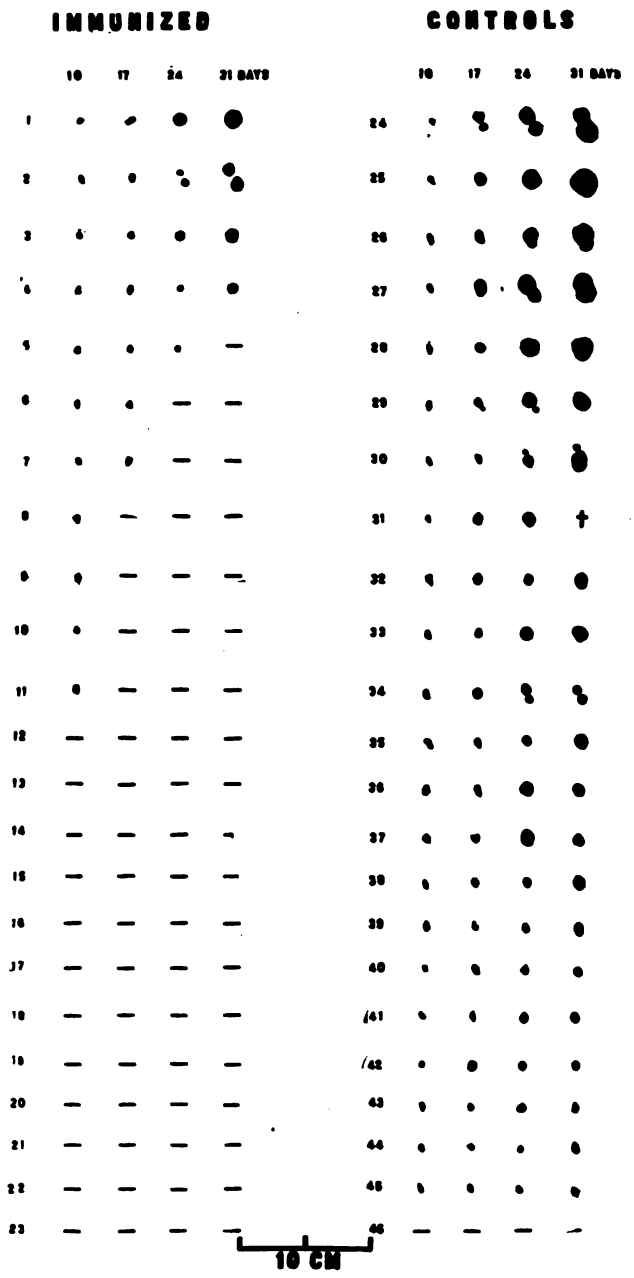

FIG. 2. EXPERIMENT $\frac{48}{33B}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

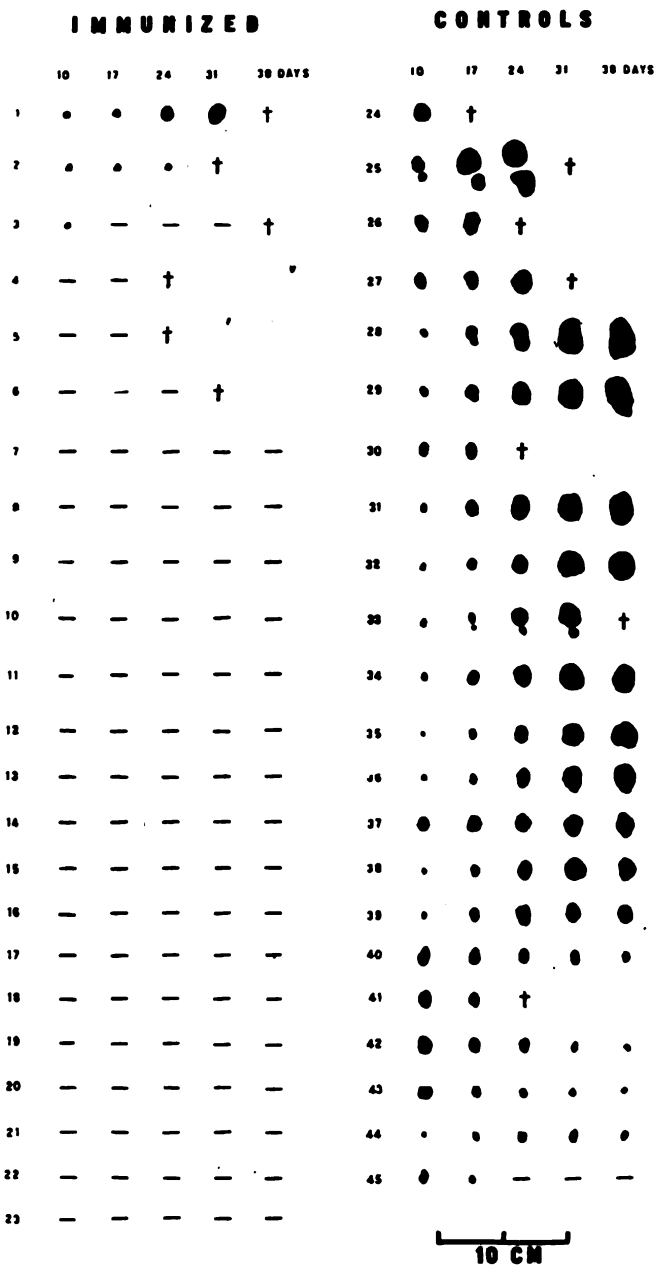

FIG. 3. EXPERIMENT $\frac{63}{136L}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

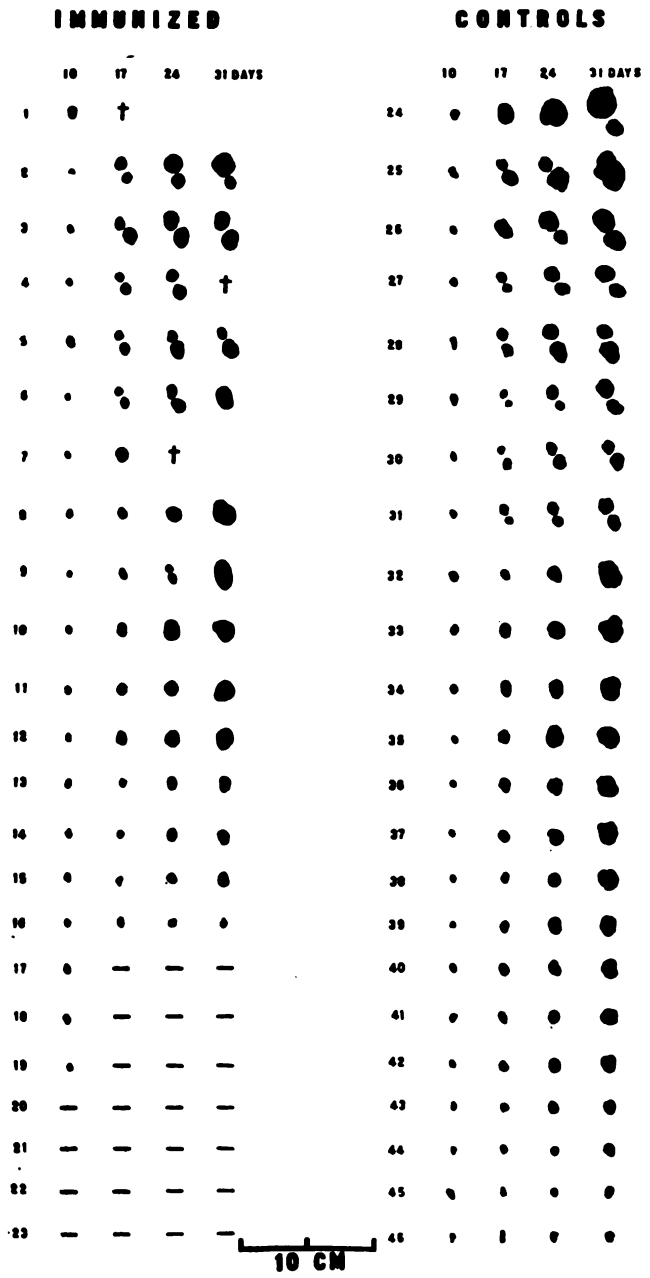

FIG. 4. EXPERIMENT $\frac{63}{141H}$

Slight immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

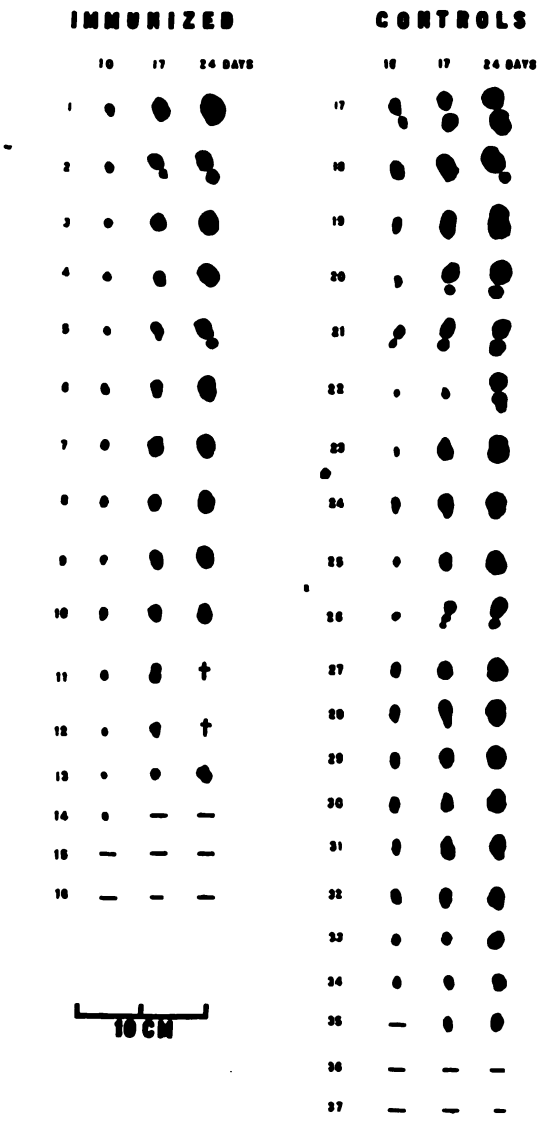

FIG. 5. EXPERIMENT $\frac{63}{138Q}$

Practically no immunity to carcinoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

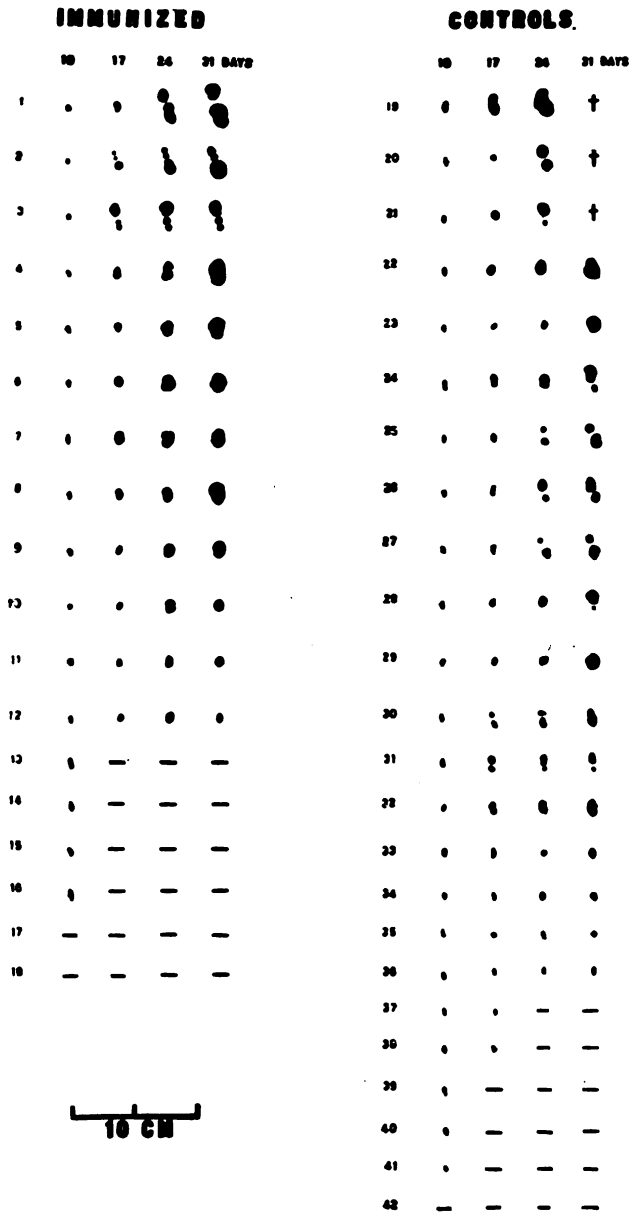

FIG. 6. EXPERIMENT $\frac{11}{44E}$

No immunity to carcinoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

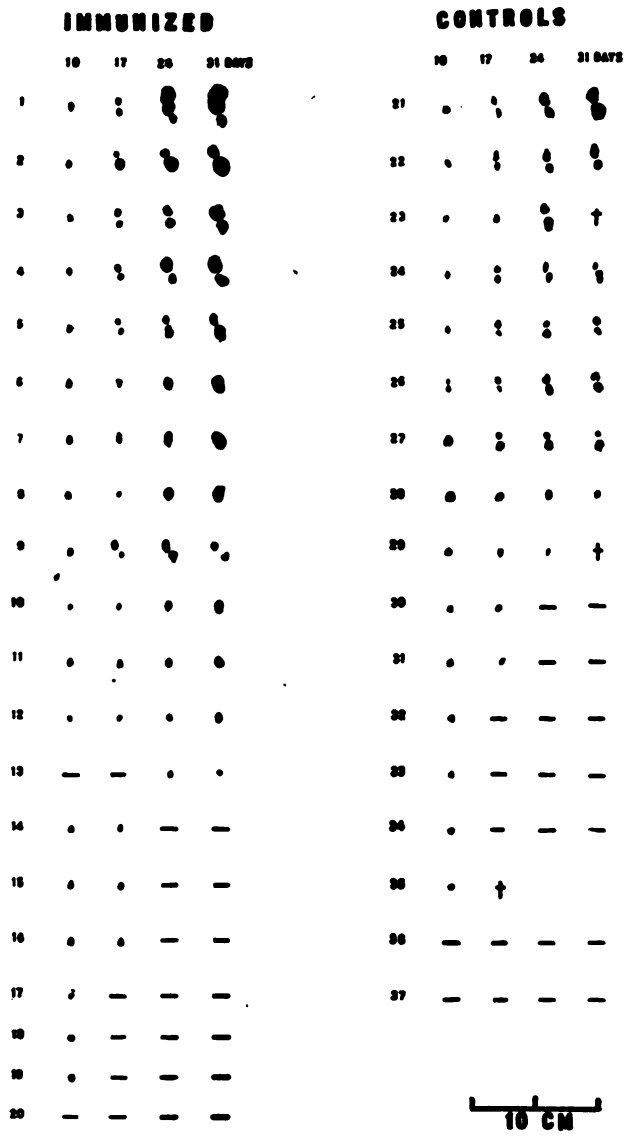

FIG. 7. EXPERIMENT $\frac{11}{44B}$

No immunity to carcinoma three weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

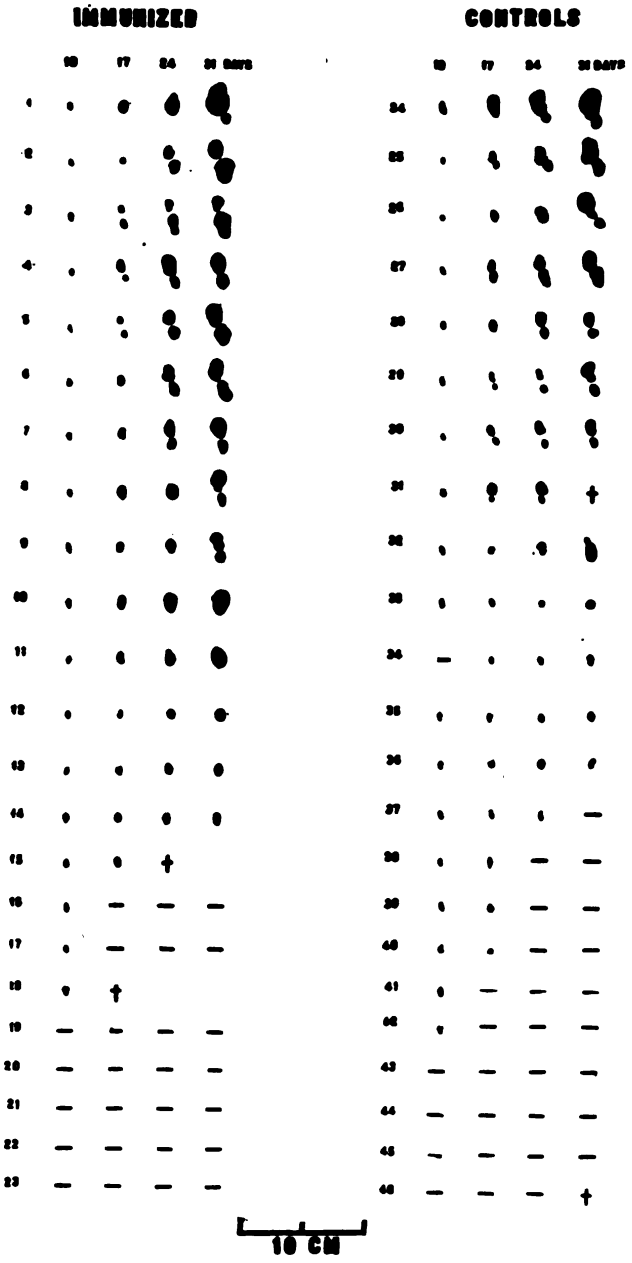

FIG. 8. EXPERIMENT $\frac{11}{44D}$

No immunity to carcinoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

skin (figs. 10 and 12, and "Treated controls" in table 2). As figures 9 to 13 and table 2 show, muscle is impotent to protect against sarcoma, and the same is true of lymph-node. In other words, the mesodermal derivatives chosen do not protect against two neoplasms of mesodermal origin, although the interval

TABLE 2

SARCOMA	INTERVAL	MICE	TREATED WITH: Muscle — Survived	Muscle — Negative — Number	Muscle — Negative — Per cent	Lymph-node — Survived	Lymph-node — Negative — Number	Lymph-node — Negative — Per cent
180	1 week	Treated	21	6	28.75	24	11	45.83
		Controls	23	1	4.34	21	2	9.52
	2 weeks	Treated	22	4	18.18	20	0	0.00
		Controls	24	0	0.00	21	0	0.00
		"Treated controls"				15	0	0.00
	3 weeks	Treated	14	1	7.14	17	2	11.76
		Controls	20	0	0.00	20	2	10.00
		"Treated controls"	22	8	36.36			
	4 weeks	Treated	33	2	6.06	11	0	0.00
		Controls	35	1	2.85	20	1	5.00
		"Treated controls"				14	1	7.14
E. S.	1 week	Treated	24	0	0.00	16	1	6.25
		Controls	19	0	0.00	19	0	0.00
		"Treated controls"	19	0	0.00			
	2 weeks	Treated	23	0	0.00	22	0	0.00
		Controls	22	1	4.58	22	1	4.58
		"Treated controls"	22	4	18.18			

between treatment and tumor inoculation was inside the limits of the period during which immunity to sarcoma can sometimes be elicited (9).

In table 3 all the tissues that have now been tested as to their immunizing power are arranged according to derivation. The failure of cartilage, bone, and muscle is not due to their meso-

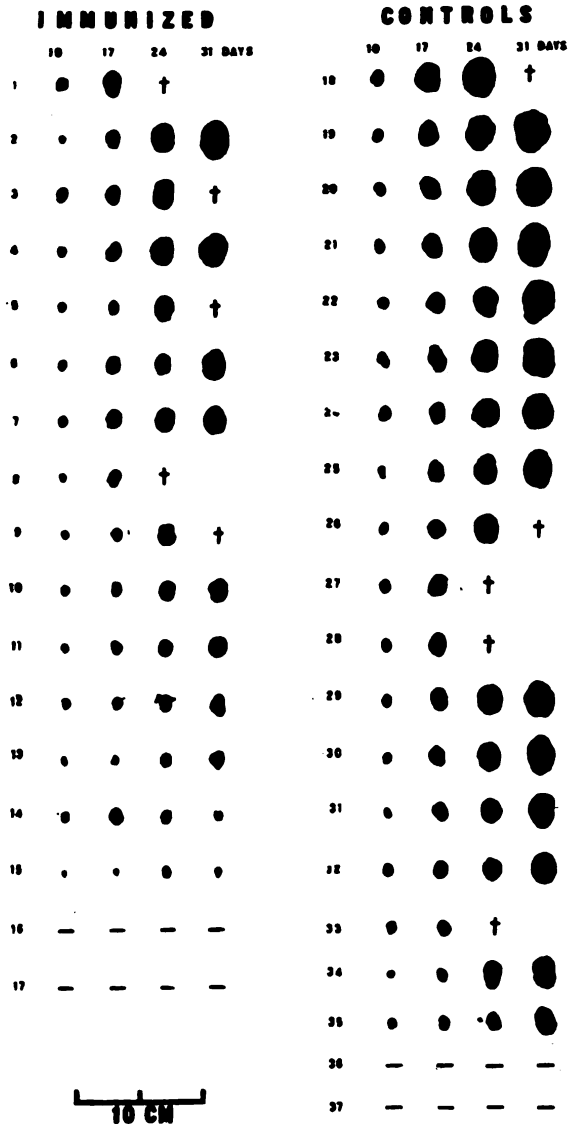

FIG. 9. EXPERIMENT $\frac{180}{26H}$

No immunity to sarcoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

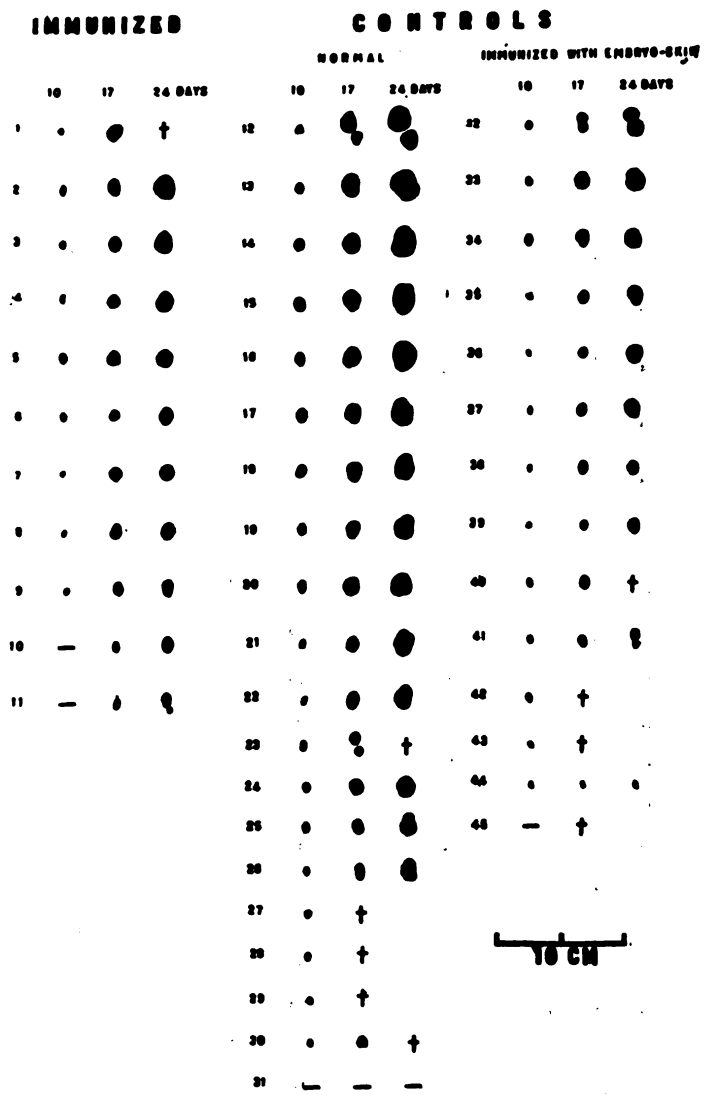

FIG. 10. EXPERIMENT $\frac{180}{431}$

No immunity to sarcoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method. It will be noted that embryo skin also fails to produce resistance against this sarcoma.

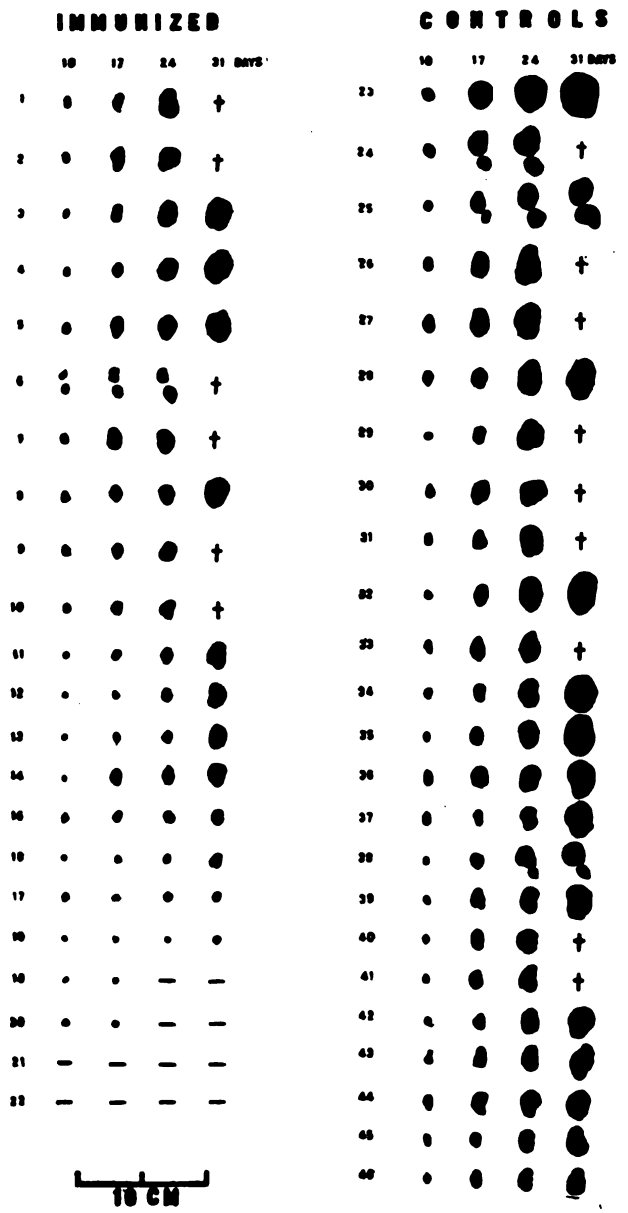

FIG. 11. EXPERIMENT $\frac{180}{26D}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

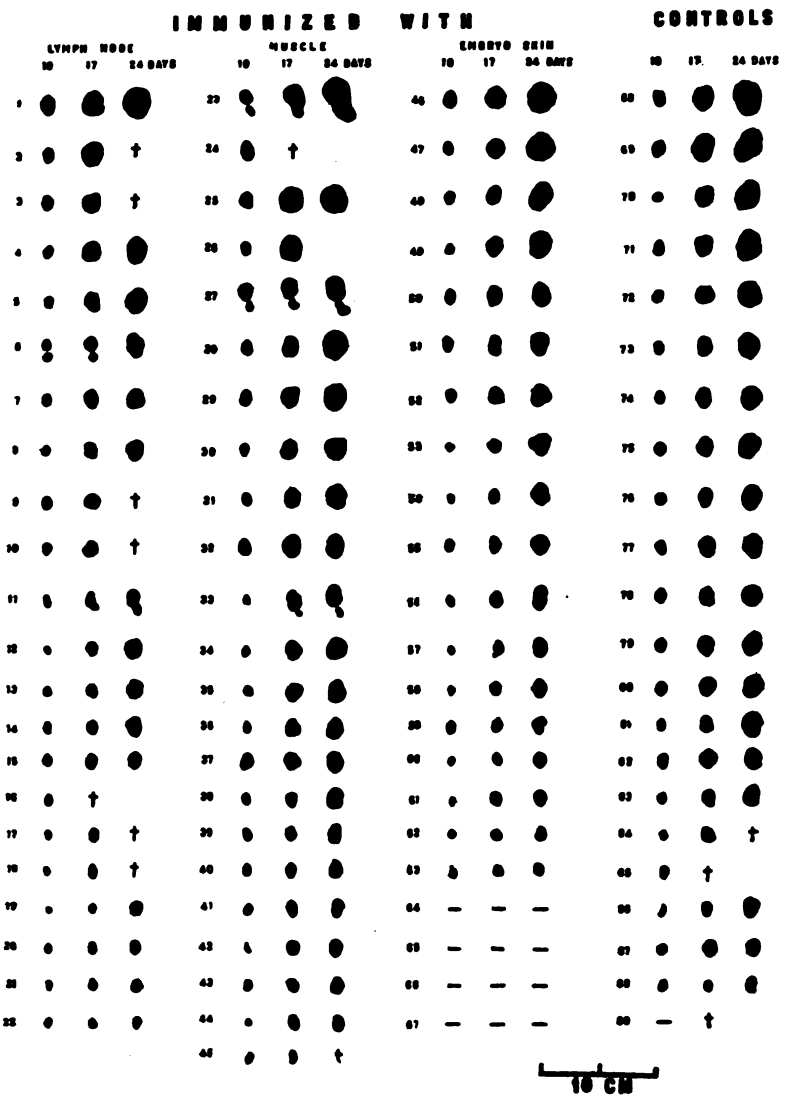

FIG. 12. EXPERIMENT $\frac{ES}{60G}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method. It will be noted that lymph-node and embryo skin also fail to produce resistance against this sarcoma.

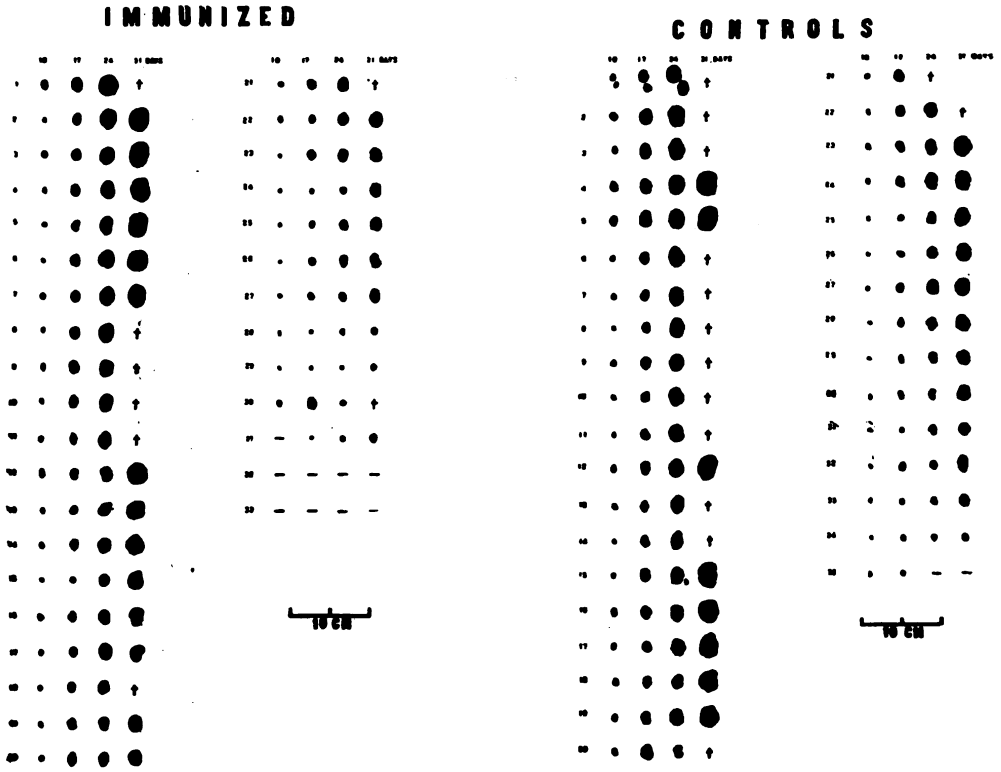

FIG. 13. EXPERIMENT $\frac{180}{26F}$

No immunity to sarcoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

dermal origin, for other materials derived from the same source are fully potent; furthermore, the inability is shared by at least one tissue descended from the ectoderm. As has already been pointed out, lens, cartilage, and bone contain comparatively few cells in contrast with those tissues which are efficient in eliciting the refractory state; yet muscle, which is more cellular, also fails completely. This can hardly be referred to its high differentiation, for the skin, which is the most active immunizer known, is perhaps as highly differentiated a tissue. The need for further investigation is obvious, and this will be

TABLE 3

Tissues which will immunize against transplantable carcinoma are followed by the plus sign

Ectoderm		Entoderm	
Fetal skin	+	Liver	+
Mamma	+		
Lens	−	All Layers	
Brain	−?		
		Embryo	+
Mesoderm			
Spleen (containing blood)	+	Extraembryonic Ectoderm and Mesoderm	
Blood	+		
Testis	+		
Kidney	+	Placenta (washed free of blood)	+
Cartilage	−		
Bone	−		
Muscle	−		
Lymph-node	+		

undertaken as soon as conditions permit; indeed, it is even now under way. The present communication, therefore, is to be regarded as scarcely more than a preliminary report.

SUMMARY

Preliminary treatment with normal tissues containing but few cells, whether they be of ectodermal or mesodermal origin, fails to induce immunity to transplantable carcinomata. Muscle, also, though this is more cellular, is inactive, for some reason at present unknown.

Lymph-node, on the contrary, has the power to elicit a high resistance against transplantable carcinomata.

The mesodermal tissues investigated have no power to immunize against two connective tissue tumors employed, failing, like the skin, to protect against sarcoma.

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